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TECHNICAL MANUSCRIPT 389

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ARTHROSPORE VACCINE  
AGAINST COCCIDIOIDOMYCOSIS IN MICE

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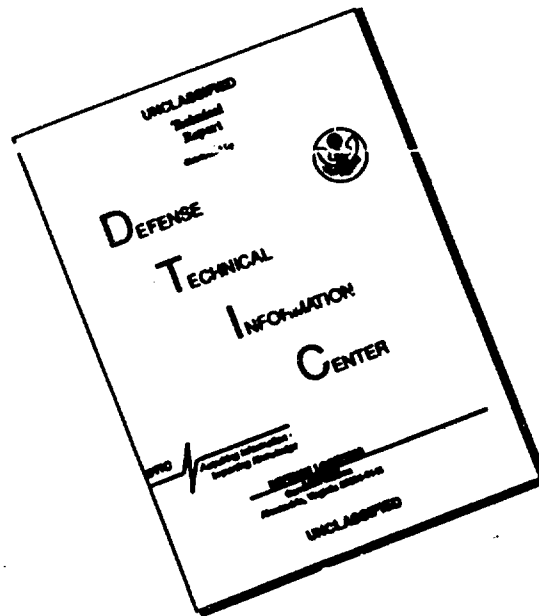
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TECHNICAL MANUSCRIPT 389

EXPERIMENTAL IRRADIATED ARTHROSPORE VACCINE  
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Project 1C522301A059

July 1967

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

#### ABSTRACT

An experimental irradiated (Co<sup>60</sup>) arthrospore vaccine against coccidioidomycosis protected approximately 75% of mice from death following an intraperitoneal challenge sufficient to kill approximately 90% of the nonimmunized control mice. Although the majority of the immunized mice became infected with Coccidioides immitis, the histologic lesions were substantially less severe than those in the nonimmunized controls, particularly in the pulmonary region. Although arthrospores irradiated with 1, 2, or 3 million roentgens lost their ability to multiply in various laboratory media (probably through interference with cell division), partial conversion to the parasitic phase (spherule) was observed after animal inoculation (rounding out of arthrospores into immature spherules, but without development of endospores). Duration of viability of these structures has yet to be determined.

## I. INTRODUCTION

The value of nonviable vaccines in experimental coccidioidomycosis has been extensively investigated in several laboratory animals. The guinea pig was used in studies by Negroni, Vivoli, and Bonfiglioli<sup>1</sup> and Vogel et al.<sup>2</sup> Friedman and Smith<sup>3</sup> and Converse et al.<sup>4</sup> used mice.

Very thorough and extensive studies in mice, comparing the parasitic and saprophytic phases of Coccidioides immitis as antigens, various routes of inoculation, and various schedules of immunization and identifying the location of immunogenicity in the structure of the organism, have been made by Levine, Cobb, and Smith,<sup>5,6</sup> Levine and Kong,<sup>7,8</sup> and Kong, Savage, and Levine.<sup>9</sup>

Similar investigations have been made by Levine, Miller, and Smith<sup>10</sup> in the cynomolgus monkey (Macaca iris), by Converse et al.,<sup>11,12</sup> Lowe et al.,<sup>13</sup> and Sinski et al.<sup>14</sup> in the rhesus monkey (Macaca mulatta), and by Castleberry et al.<sup>15</sup> in the dog.

Finally, the tissue reaction to an experimental vaccine of this type (formalin-killed C. immitis spherules) has been studied in human volunteers by Levine and Smith<sup>16</sup> and Pappagianis, Levine, and Smith.<sup>17</sup>

All of the animal studies (excluding the human trials) have shown that a nonviable vaccine, by modification of the disease, extends the survival time and decreases the severity of the lesions, but does not prevent infection. Although surviving animals appear outwardly healthy, they retain the organism in the tissues in a viable state for a long time. Pappagianis et al.<sup>18,19</sup> and Converse et al.,<sup>4,11,12</sup> using monkeys (M. iris and M. mulatta) and mice, and Castleberry et al.,<sup>20</sup> using dogs, have shown that experimental viable C. immitis vaccines are far superior to nonviable vaccines in their protective effect against infection.

The purpose of this study was to investigate the efficacy of an irradiated C. immitis vaccine. It has been shown that mammalian tissue cell cultures as well as certain bacteria and fungi lose their ability to divide after exposure to certain levels of radiation. These cells can, however, maintain viability for a long time.<sup>21</sup> If irradiated C. immitis behaves in a like manner, it might more nearly approach the immunogenicity of a viable vaccine without the attending danger of extensive multiplication after injection.

## II. MATERIALS AND METHODS

### A. VACCINE STRAIN

*C. immitis*, strain Cash, an isolate from a nonfatal disseminated human infection, obtained from Dr. C.E. Smith, University of California, Berkeley, was grown as previously described<sup>4</sup> in the synthetic medium of Roessler et al.<sup>22</sup> The harvested arthrospores (approximately 98% unipartulate suspension) were concentrated by removal of the culture supernatant or by resuspension in 8% aqueous glucose to suspensions containing 12 mg per ml. These suspensions were stored at 5 C both before and after irradiation.

### B. IRRADIATION OF VACCINES

Samples of each suspension were subjected to irradiation (gamma source: Co<sup>60</sup>) of 100,000, 200,000 or 300,000 or 1, 2, or 3 million r. Arthrospores suspended in glucose were kept frozen (-20 to -10 C) during irradiation; those in the culture supernatant were maintained at approximately 5 C during irradiation.

### C. POSTIRRADIATION EXAMINATION OF VACCINES

Plate counts of appropriate dilutions were made on GPY agar (1% glucose, 2% peptone, 0.1% yeast autolyzate) after irradiation to determine ability to undergo cell division. Mice (18 to 24 g Swiss-Webster) received two 0.25-ml intraperitoneal (IP) injections 1 week apart of all vaccines failing to multiply on agar or in the liquid synthetic medium of Roessler et al.<sup>22</sup> They were then examined histologically for evidence of infection 90 days postinoculation.

### D. IMMUNIZATION AND CHALLENGE

Eighteen- to 24-g white Swiss mice (approximately 25 mice per group) received two 0.25-ml IP injections (total dose, 7 mg of vaccines subjected to 1, 2, or 3 million r of gamma radiation at an interval of 7 days. Two weeks later an IP challenge dose (1,300 viable strain Cash arthrospores, grown as stated above) was administered.

### E. PATHOGENESIS

All mice were observed for 90 days, dates of deaths were recorded, and all survivors were autopsied. The findings on gross examination were recorded. Tissues for histopathological studies were prepared in the usual manner. Sections were stained with the Giemsa, PAS, and Gomori methenamine silver nitrate stains.



## F. CLASSIFICATION OF HISTOPATHOLOGICAL FINDINGS

Histopathological findings were classified as - to +++, as follows:

- = No lesions referable to C. immitis were seen.
- + = A few discrete granulomas, less than 1 mm in diameter. The lesions were characterized by an infiltrate of histiocytes, epithelioid cells, plasma cells, lymphocytes, and occasionally polymorphonuclear leukocytes. Spherules of C. immitis were not seen.
- ++ = Same as +, but with identifiable spherules of C. immitis.
- +++ = 1- to 3-mm lesions, usually with central caseation or liquefaction necrosis. Spherules were numerous in the lesions.
- ++++ = Multiple nodules, the majority of which were greater than 3 mm or confluent. Central caseation or liquefaction necrosis and spherules of C. immitis were abundant. Extensive dissemination and contiguous involvement of adjacent serosal surfaces and organs often present.

## III. RESULTS

### A. EFFECT OF IRRADIATION ON C. IMMITIS

As shown in Figure 1, 100,000 r of gamma radiation resulted in 95% inactivation of arthrospores. Minimal reproduction was evident at 200,000 and 500,000 r, with approximately 10 colonies out of  $3 \times 10^8$  organisms at the 500,000-dose level. No growth was noted in cultures of arthrospores irradiated with dose levels of 1, 2, or 3 million r. No difference was noted between the inactivation curves of arthrospores irradiated in the frozen state and of those irradiated at approximately 5 C.

### B. MORTALITY OF CHALLENGED ANIMALS

No significant difference in mortality (21 to 28%) was noted among any of the vaccinated groups, regardless of radiation dose of the antigen or state of antigen (frozen or unfrozen) during irradiation. As seen in Figure 2, 77% of the vaccinated animals survived for 3 months after challenge. In contrast, 16 of the 18 nonimmunized, challenged, control animals (89%) died from 13 to 88 days after challenge; the two surviving control animals showed severe pulmonary and abdominal lesions upon autopsy at 90 days.

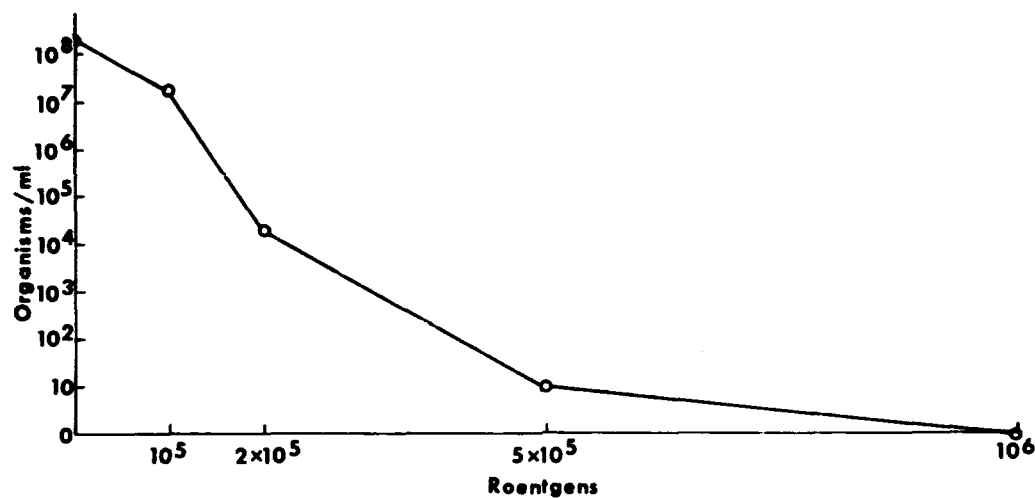


Figure 1. Inactivation Curve for *Coccidioides immitis* Arthrospores Irradiated with Gamma Rays (Source, Cobalt 60). No cell division noted at doses of 1, 2, or 3 million r.

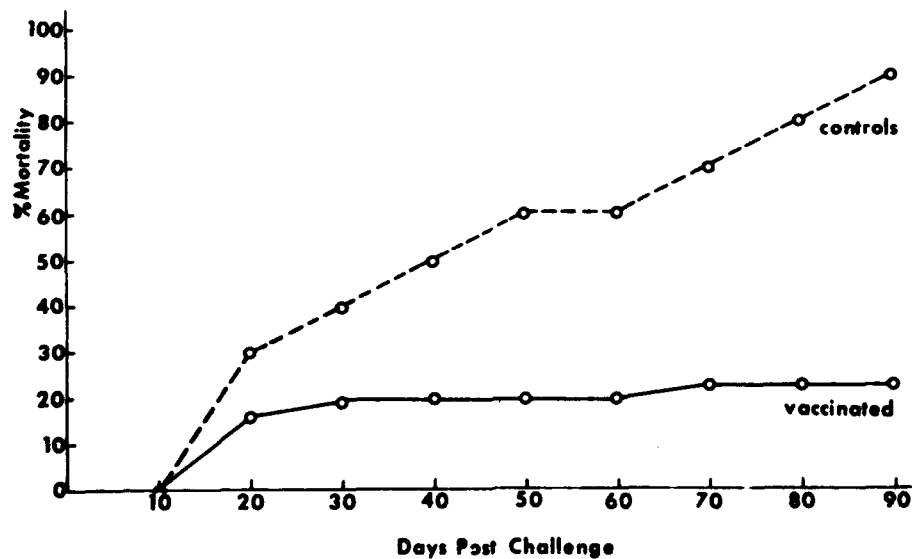


Figure 2. Mortality Curve for Mice Vaccinated with Irradiated (Co<sup>60</sup>) *Coccidioides immitis* Arthrospores and Challenged with 1,300 Virulent Arthrospores. Dotted line shows mortality of nonvaccinated control mice.

### C. PATHOLOGICAL FINDINGS

Upon autopsy of the challenged animal gross evidence of infection was negligible in the immunized survivors, greater in immunized animals dying of the infection, and most extensive in the nonimmunized control animals. Gross pathology in the nonchallenged, immunized controls was practically nonexistent.

Histological examination of the challenged animals (Table 1) revealed that the organs most commonly affected in immunized animals surviving IP challenge were the spleen and the liver. The main difference between surviving animals and those dying from infection was more frequent involvement of the lung, kidney, and pancreas in the latter. Moreover, death was usually associated with extensive lung destruction (Fig. 3).

TABLE 1. DISTRIBUTION OF LESIONS IN MICE AFTER INTRAPERITONEAL CHALLENGE WITH COCCIDIODES IMMITIS

Group	Number of Animals	Per Cent of Total with Involvement				
		Spleen	Liver	Pancreas	Kidney	Lung
Immunized and survived	70	64	37	21	16	23
Immunized and died	24	92	91	86	53	95
Nonimmunized controls	18	100	100	75	86	100

In general, + to ++ lesions were found in survivors, compared with +++ to ++++ lesions in animals dying from the infection (Table 2). The greatest difference between immunized and nonimmunized animals was the lower proportion of pulmonary lesions in those receiving the vaccine (41% vs. 100% for controls). A characteristic abdominal lesion in the vaccinated animals (graded ++) consisted of a chronic inflammatory infiltrate with prominent macrophages. The spherules and endospores were frequently seen in the granular eosinophilic cytoplasm of the cells. Such foci were adjacent to organ capsules without distinct parenchymal or, less often, capsular infiltration (Fig. 4). This type of lesion was not seen in nonimmunized animals. Parenchymal organ involvement was characteristic of the nonimmunized group. Minimal lesions, generally confined to the splenic or hepatic capsules, were noted upon histologic examination of the abdominal organs of the nonchallenged, immunized controls (32 animals). Although the organism was noted in many of these

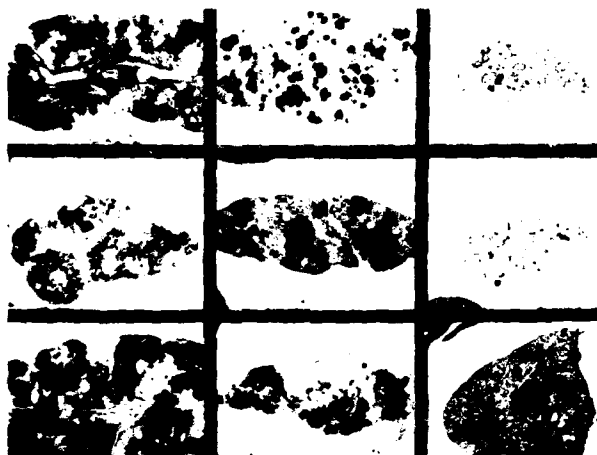


Figure 3. Representative Sections of Lung Tissue.  
 A. Nonvaccinated, challenged control mice.  
 B. Vaccinated mice dying from the challenge dose.  
 C. Vaccinated mice surviving the challenge dose.  
 Note massive lung destruction in the control group (A) compared with the minimal changes in the survivors (C).

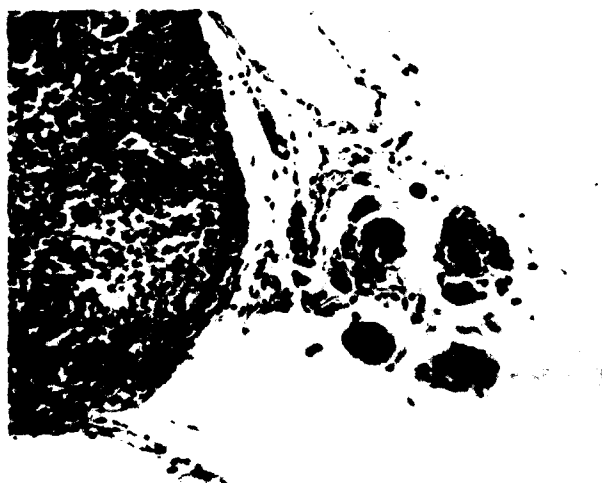


Figure 4. Characteristic + or ++ Abdominal Lesion seen in Vaccinated Challenged Mice, Adjacent to Organ Capsules without Parenchymal or Capsular Infiltration.

(Fig. 5B), a difference in its development was obvious. The arthrospores from the irradiated vaccine appeared to have rounded into typical, immature spherules, but there the development had stopped. No endospores were seen in any of the spherules noted in the nonchallenged, immunized control group.

TABLE 2. SEVERITY OF LESIONS IN MICE AFTER IP CHALLENGE WITH COCCIDIODES IMMITIS

Group	Number of Animals	Classification of Lesions	
		+ and ++, %	+++ and +++++, %
Immunized and survived	70	68	32
Immunized and died	24	45	55
Nonimmunized controls	18	26	74

#### IV. DISCUSSION

Although a large majority of the vaccinated animals survived for 3 months after a massive challenge with C. immitis, only 10 to 20% remained free of infection. This was similar to results obtained previously with a formaldehyde-killed vaccine.<sup>4</sup> However, histological changes in this study were less marked than those observed with the formaldehyde-killed vaccine.

A prognostic evaluation of the changes in the sacrificed animals is necessarily arbitrary, but it is probable that lesions graded ++ or less would have resolved. The difference between + and ++ lesions was not considered significant. The lesions graded + (those without organisms present) could not be definitely attributed to infection by C. immitis, although they were compatible with those produced by coccidioidomycosis and probably were caused by the organism; histologic identification of organisms in minimal lesions is often somewhat fortuitous. Even if we consider a + lesion diagnostic, the scarcity of organisms could be interpreted as resistance to infection due to vaccination.

The fact that foci of infection in the vaccinated animals were often confined to serosal surfaces without parenchymal infiltration is additional evidence of defense by the immunized host.

Although arthrospores irradiated with Co60 in the 1- to 3-million range were unable to divide, it appears that they were still viable at the time of inoculation, because partial conversion of arthrospores to the parasitic phase (Fig. 5) was obvious in animal tissue from the nonchallenged vaccinated control animals. The lack of endosporulation in these spherules was attributed to an inability of cells to divide.

The preliminary study reported here indicates the potential value of an irradiated vaccine against coccidioidomycosis. However, a further comparison of irradiated vaccines and formaldehyde-killed vaccines (both arthrospore and spherule) in monkeys challenged via the respiratory route might provide a more satisfactory indication of its worth.

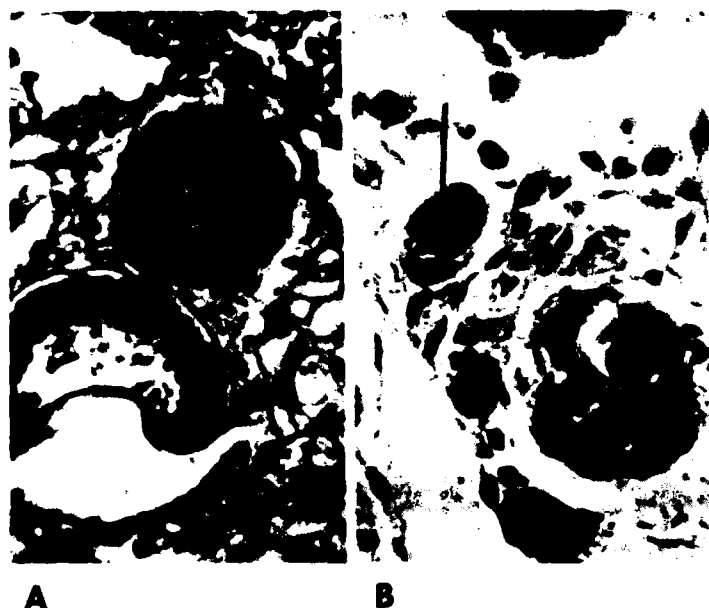


Figure 5. Comparison of *Coccidioides immitis* Spherules Developing from (A) Nonirradiated Arthrospores and (B) Irradiated Arthrospores. Note presence of endospores in the former and the much smaller, rounded-out, immature spherule (arrow) in the latter. 450X.

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